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Value and feasibility of screening women attending STD clinics for cervical chlamydial infections

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SUMMARY A chlamydial screening service was provided in Bristol over a three-month period for women attending the sexually transmitted disease (STD) clinic either for the first time or with a new complaint. Isolation of *Chlamydia trachomatis* was attempted in cytochalasin-treated McCoy cells. Of 919 specimens, valid results were obtained in 796. Chlamydial infections were identified and treated in 154 (19%) of these 796 women. Chlamydia were isolated from 52 (37%) of 152 female partners of men with nongonococcal urethritis (NGU); these patients already routinely receive treatment with tetracyclines in this clinic. The remaining 102 infections (34 (48%) of 71 women with gonorrhoea and 68 (12%) of the other 573 women) would have been unrecognised and usually untreated without chlamydial isolation studies. These figures confirm the need to provide chlamydial diagnostic services for selected STD clinic attenders.

Introduction

Patients attending sexually transmitted disease (STD) clinics are frequently infected with genital strains of *Chlamydia trachomatis.* These obligate intracellular bacterial parasites are sexually transmitted organisms, which are now recognised as an important cause of non-specific genital infections.[1-3] Definitive diagnosis of a genital chlamydial infection depends on isolation of the organism in in-vitro cell cultures, but since existing techniques are time-consuming and labour-intensive, it is difficult at present to provide routine screening of patients attending STD clinics on the scale now available for the diagnosis of infections with *Neisseria gonorrhoeae, Trichomonas vaginalis,* and *Candida albicans.*

Shedding of chlamydia from the male urethra is usually associated with the symptoms and signs of non-specific urethritis, which may present as either nongonococcal or post-gonococcal urethritis, for which the patient routinely receives treatment with tetracyclines. Chlamydia are sensitive to these antibiotics,[4 5] and therefore, whether or not laboratory facilities for the isolation of chlamydia are available, men with active urethral chlamydial infections usually receive appropriate antimicrobial therapy. In women, however, shedding of chlamydia from the cervix is not associated with distinct clinical features.[6-8] Moreover, apart from the female partners of men with nongonococcal urethritis (NGU) who are now frequently given epidemiological treatment on the basis of their partner's urethritis, women attending STD clinics do not routinely receive tetracyclines. Many cervical chlamydial infections therefore go both unrecognised and untreated.

In an attempt to identify these cervical infections, a chlamydial screening service was provided for all new female patients who presented at the STD clinic of the Bristol Royal Infirmary for a three-month period. The purpose of this study was to assess the feasibility of providing such a laboratory service and to establish the practical relevance of such a service to the clinician.

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Patients and methods

STUDY GROUP

Patients consisted of women who attended the STD clinic at the Bristol Royal Infirmary for the first time between 19 February 1979 and 25 May 1979 and women who had attended the clinic previously but who presented during this period with a new complaint. Retrospectively patients were assigned to one of three categories: (a) those from whom *N gonorrhoeae* was isolated at the time they were screened for chlamydia; (b) those identified from their medical records as consorts of men with NGU; and (c) the remainder of the study group.

SPECIMENS FOR CHLAMYDIAL ISOLATION

In addition to the specimens routinely collected from all patients for the diagnosis of infections with *N gonorrhoeae, T vaginalis,* and *C albicans*,[6] an endocervical swab for chlamydial isolation was obtained from each patient by a member of the nursing or medical staff and placed in sorbitol transport medium.[9] Each specimen was held at 4°C until transported to the laboratory where it was either inoculated into cell cultures or snap frozen at −70°C within 24 hours of being taken. Specimens were delivered to the laboratory each weekday morning and early afternoon, and on Friday evenings an additional delivery of specimens obtained at the evening clinic was made. Clinicians were asked to state whether or not the patient had received any antimicrobial therapy during the previous month.

CELL CULTURE TECHNIQUE

Isolation of *C trachomatis* was attempted in McCoy cells treated with cytochalasin B.[10] With this technique McCoy cells are treated with cytochalasin for three days before inoculation of the clinical specimen. Each Tuesday and Friday approximately 50 culture tubes (Nunc, Code 3-62707, Gibco-Biocult) containing a sterile circular glass coverslip 10 mm in diameter were each seeded with $1{\cdot}2 \times 10^5$ McCoy cells in 1 ml growth medium containing 1 μg cytochalasin and incubated at 35°C for three days. Cultures made on Friday were either inoculated on Monday or held at room temperature for 24 or 48 hours for inoculation of specimens received on Tuesdays and Wednesdays. Cultures made on Tuesday were inoculated on Friday. Cultures inoculated without being held at room temperature for 24 or 48 hours are subsequently referred to as "fresh" cultures. All specimens which arrived when cell cultures were available were inoculated within 24 hours of being taken but specimens received at times when cultures were not available were snap frozen and stored at −70°C until inoculation.

Each specimen was inoculated into one cell culture (0·25 ml inoculum) and then stored at −70°C until an unequivocal result was obtained. Inoculated cultures were centrifuged at 3000 × *g* for one hour at 34°C and incubated at 35°C for about one hour. The growth medium and inoculum from each tube was then replaced with 1 ml maintenance medium containing 1 μg cytochalasin and cultures were reincubated for a further two or three days. Cell monolayers were then fixed in methanol, stained with Giemsa, and scanned for typical intracytoplasmic inclusions. If an equivocal result was obtained from a specimen it was cultured again.

When monolayers yielded chlamydial inclusions the number of inclusions per coverslip were assessed by scanning the whole monolayer, when $\leqslant 10^3$ inclusions were present, and by counting inclusions in a proportion of the monolayer, when $>10^3$ inclusions were present.

STATISTICAL ANALYSIS

The significance of the findings in this study was assessed by the χ^2 test.

Results

Specimens were obtained from 919 patients. Processing these specimens occupied one of us (IDP) full-time and one of us (SJR) for 4-8 hours a week, the latter time being spent mainly on the microscopy work. It was usually possible to report the results within 5-8 days of receiving a specimen, the delay (apart from the necessary 2-3 days' incubation) being due largely to the time taken to scan large numbers of preparations by darkground microscopy.

Of the 919 specimens, 10 were inadvertently left at 4°C for 72 hours, 14 were contaminated or were toxic to the cells, and 99 were from patients who had received antimicrobial therapy during the previous month. Valid results were therefore obtained on 796 specimens; only five of these specimens had to be cultured twice before an unequivocal result was obtained.

COMPARISON OF CULTURE TECHNIQUES

"Holding" before inoculation

Chlamydia were isolated from 37 (19%) of 196 unfrozen specimens inoculated into fresh cytochalasin-treated McCoy cell cultures compared with 35 (23%) of 155 unfrozen specimens inoculated into cultures held at room temperature for 24 hours and 14 (15%) of 95 unfrozen specimens inoculated into cultures held at room temperature for 48 hours (Table I). The difference between these isolation rates was not statistically significant ($\chi^2_2 = 3{\cdot}574$, $0{\cdot}1<P<0{\cdot}2$) indicating that cultures kept at room temperature for 24 or 48 hours before inoculation are

TABLE I *Results of chlamydial isolation in fresh McCoy cell cultures and in cultures held at room temperature for 24 or 48 hours before inoculation*

Cytochalasin-treated McCoy cell cultures	*Positive specimens out of total tested†*	
	No	*%*
Fresh*	37/196	19
Held at room temperature for		
24 hours	35/155	23
48 hours	14/95	15

*Cell cultures inoculated after three-day treatment with cytochalasin
†Unfrozen specimens

as satisfactory for growth of chlamydia as fresh cultures.

Freezing before inoculation

Storage of specimens at −70°C before inoculation had no effect on the chlamydial isolation rate. Thus chlamydia were isolated from 68/350 (19%) of frozen specimens compared with 86/446 (19%) of unfrozen specimens. Moreover, when chlamydia were isolated, the yields of inclusions from the frozen and unfrozen specimens were similar, indicating there had been no loss of infectivity in the frozen specimens (Table II). Overall, 40 (26%) of 154 positive specimens yielded <10 inclusions/coverslip and 30 (19·5%) specimens yielded $>10^3$ inclusions/coverslip.

ISOLATION IN WOMEN WITH GONORRHOEA AND IN FEMALE PARTNERS OF MEN WITH NGU

N gonorrhoeae was cultured from 71 patients at the time specimens for isolation of chlamydia were obtained, and chlamydia were recovered from 34 (48%) of these patients. One hundred and forty-two patients were partners of men with NGU and chlamydia were isolated from 52 (37%) of these women. Chlamydia were isolated from 68 (12%) of the remaining 573 patients.

MANAGEMENT OF CHLAMYDIAL INFECTIONS

All patients from whom *C trachomatis* was recovered were treated with a course of tetracyclines (normally oxytetracycline 1 g/day for 10 days). All women who were partners of men with NGU received treatment before the chlamydial isolation result was known; most other patients infected with chlamydia received their treatment as a result of the laboratory report.

Discussion

Various modifications of the technique of isolating chlamydia in cytochalasin-treated McCoy cells enabled a large number of specimens (60-80/week) to be tested in this study. By holding cell cultures at room temperature for 24-48 hours before inoculation it was necessary to prepare cell cultures only twice a week; inoculation of clinical specimens into the growth medium of the cell cultures and subsequent replacement of both inoculum and growth medium with maintenance medium after centrifugation produced monolayers of good quality which were easy to scan by darkground microscopy and which rarely yielded equivocal results; the use of one rather than two cell cultures for each specimen halved the number of cultures required, while storage of clinical specimens at −70°C without loss of infectivity meant that a particular isolate could be retrieved and passaged in McCoy cells if this was necessary for serotyping or other purposes.

Previous studies have shown consistently that chlamydia are isolated significantly more often from women with gonorrhoea and from women who are consorts of men with NGU than from other women attending STD clinics.[6 7 11-13] In these earlier studies infections with *T vaginalis* and *C albicans*—organisms which predominantly infect the vagina rather than the endocervix—had no significant effect on the recovery of chlamydia. The chlamydial isolation rates in women infected with *T vaginalis* and *C albicans* have therefore not been analysed in this present study. The isolation rates of 37% in consorts of men with NGU, of 48% in women with gonorrhoea, and of 12% in the remainder of the women studied are comparable to those reported in the earlier studies.

Overall the isolation rate was lower in the present study than in that reported previously from the same clinic.[6] This was probably due partly to the fact that in the earlier study 20% of the women had gonorrhoea compared with only 9% in the present survey. Since women with gonorrhoea are so frequently infected with chlamydia, reduction in the rate of gonococcal isolation in a given group of women would also be associated with a lower chlamydial isolation rate. In addition, the chlamydial isolation rate in women with gonorrhoea was lower in the present study (48%) than that reported earlier (63%), although this difference was not statistically significant ($\chi^2 = 2 \cdot 39$, $0 \cdot 1 < P < 0 \cdot 2$). Irradiated

TABLE II *Comparison of yields of inclusions from positive specimens stored at −70°C and positive specimens kept at 4°C for 24 hours or less*

	No of positive specimens (%)				
	*Inclusions/coverslip**				
	<10	*10-99*	*100-999*	*>1000*	*Total*
Stored at −70°C	18 (26)	19 (28)	15 (22)	16 (24)	68 (100)
Kept at 4°C for <24 hr	22 (26)	29 (34)	21 (24)	14 (16)	86 (100)
Total	40 (26)	48 (31)	36 (23·5)	30 (19·5)	154 (100)

*Each culture inoculated with 0·25 ml of clinical specimen

McCoy cells rather than cytochalasin-treated McCoy cells were used in the earlier study; however, the range of inclusion counts, in particular the proportion of positive specimens yielding $>10^3$ inclusions per coverslip, was very similar in the two studies (20% in 1974 compared with 19·5% in the present work) indicating that the sensitivity of the two isolation procedures was similar.

There is convincing evidence at present that not only are genital chlamydia pathogens in the male urethra and cervix but that by ascent of the genital tract or by transfer to the eyes and respiratory tract they can also cause serious disease elsewhere.[14-20] Identification and eradication of these infections, particularly the large reservoir of infection in the genital tract, is therefore undoubtedly desirable. The present work allowed 158 genital chlamydial infections to be identified and treated during the three-month study period. Without isolation studies these infections would have been unrecognised, and, in women who were not partners of men with NGU, they would have remained untreated. Since this study ended, chlamydial isolation efforts in this clinic have therefore been concentrated on the latter group of patients.

This study confirms the high prevalence of chlamydial infections in women with gonorrhoea as well as in female partners of men with NGU; in a given group of women the overall chlamydial isolation rate will be significantly influenced by the proportion of these women in the total group. This study also raises the question of whether or not male partners of women with cervical chlamydial infections, not known to have non-specific urethritis, should be traced and treated with tetracyclines on epidemiological grounds.

Although present cell culture techniques for isolation of chlamydia are too cumbersome to allow mass screening of the large numbers of patients who presently attend STD clinics in the UK, screening of selected groups of patients is both feasible with limited laboratory facilities and of value in identifying and eradicating genital chlamydial infections which would not otherwise be treated.

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References

1. Schachter J. Chlamydial infections (first of three parts). *N Engl J Med* 1978; **298:** 248-435.
2. Schachter J. Chlamydial infections (second of three parts). *N Engl J Med* 1978; **298:** 490-5.
3. Richmond SJ, Oriel JD. Recognition and management of genital chlamydial infection. *Br Med J* 1978; **2:** 480-3.
4. Blackman HJ, Yoneda C, Dawson CR, *et al.* Antibiotic susceptibility of *Chlamydia trachomatis. Antimicrob Agents Chemother* 1977; **12:** 673-7.
5. Ridgway GL, Owen JM, Oriel JD. The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture. *Br J Vener Dis* 1978; **54:** 103-6.
6. Hilton AL, Richmond SJ, Milne JD, *et al. Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974; **50:** 1-10.
7. Nayyar KC, O'Neill JJ, Hambling MH, *et al.* Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1976; **52:** 396-8.
8. Oriel JD, Johnson AL, Barlow D, *et al.* Infection of the uterine cervix with *Chlamydia trachomatis. J Infect Dis* 1978; **137:** 443-51.
9. Richmond SJ. The isolation of *Chlamydia* subgroup A (*Chlamydia trachomatis*) in irradiated McCoy cells. *Med Lab Technol* 1974; **31:** 7-9.
10. Sompolinsky D, Richmond SJ. Growth of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin B. *Appl Microbiol* 1974; **28:** 912-4.
11. Oriel JD, Powis PA, Reeve P, *et al.* Chlamydial infections of the cervix. *Br J Vener Dis* 1974; **50:** 11-16.
12. Burns DCM, Darougar S, Thin RN, *et al.* Isolation of *Chlamydia* from women attending a clinic for sexually transmitted disease. *Br J Vener Dis* 1975; **51:** 314-8.
13. Ridgway GL, Oriel JD. Interrelationship of *Chlamydia trachomatis* and other pathogens in the female genital tract. *J Clin Path* 1977; **30:** 933-6.
14. Beem MO, Saxon EM. Respiratory tract colonisation and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis. N Engl J Med* 1977; **296:** 306-10.
15. Harnisch JP, Berger RE, Alexander ER, *et al.* Aetiology of acute epididymitis. *Lancet* 1977; **1:** 819-21.
16. Mårdh P-A, Ripa T, Svensson L, *et al. Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977; **296:** 1377-9.
17. Rees E, Tait IA, Hobson D, *et al.* Perinatal chlamydial infections. In: Hobson D, Holmes KK (eds). *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 140-7.
18. Schachter J. The expanding clinical spectrum of infections with *Chlamydia trachomatis. Sex Transm Dis* 1977; **4:** 116-8.
19. Paavonen J, Saikku P, Vesterinen E, *et al. Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979; **55:** 203-6.
20. Tipple MA, Beem MO, Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. *Pediatrics* 1979; **63:** 192-203.